FINAL REPORT

Microbial Safety: Rapid Methods for Shellfish and Seawater - Injured E. coli

George W. Chang, Associate Professor
University of California, Berkeley
NA47FD0416
August 1, 1994 to January 31, 1996

ABSTRACT

This study addressed two major problems: (1) Development of a simple, rapid, sensitive test for fecal contamination in SHELLFISH based the indole production by Escherichia coli, and (2) Need for a reliable test for fecal contamination in SEAWATER. We found that our "Colitag-S" medium and its indole reaction gave us an excellent MPN test for E.coli in shellfish. It supplies trimethylamine that neutralizes acid produced from glycogen in shellfish tissue, permitting the inclusion of large amounts of shellfish in each culture tube. This increases sensitivity. As for resuscitating seawater-injured E.coli for use as a seawater fecal indicator, we found that pyruvate in the medium would greatly enhance the recovery of E.coli injured under laboratory conditions. However preliminary studies with field samples were less than promising.

EXECUTIVE SUMMARY

The indole test of "Colitag-S" medium offers a simple, rapid, sensitive, and specific MPN test for the fecal indicator bacterium Escherichia coli in shellfish. Attempts to improve resuscitation of seawater-injured E.coli and thus to make it a more suitable fecal indicator in marine waters were promising in the laboratory. However, preliminary field studies were less positive.

PURPOSE

A. Description of the problems.

Detection of fecal contamination in SHELLFISH is very important for food safety. However, it has always been a slow, cumbersome process. Furthermore, detection of fecal contamination of marine WATERS has problems of its own. E.coli, the widely used fecal indicator, seems to die off too rapidly in seawater, leading to a false sense of security.

B. Objective.

1. Devise and validate a simple, rapid test for E.coli in shellfish.
2. Explore ways of resuscitating seawater-injured E.coli, thus making the bacterium a more useful fecal indicator in the marine environment.

APPROACH

Test for E.coli in shellfish. After many preliminary studies, we developed "Colitag-S" medium. This medium contains buffer salts, protein hydrolysate, tryptophan (to assure indole production by E.coli), and trimethylamine oxide (TMAO, which E.coli and related bacteria convert to trimethylarnine, TMA, a basic product that will neutralize acids produced from the fermentation of endogenous shellfish glycogen). This medium is

mixed with shellfish tissue and incubated for 4 hr at 35 degrees, followed by 20 hr at 44.5 degrees. Then indole production is assessed by the addition of a specific color reagent. Many samples of shellfish were analyzed by both the one-day "Colitag-S" method and the two-to-three-day standard method, which involves two media at two different temperatures (LTB at 35 deg, and EC+MUG at 44.5 deg.). At one time we noticed that different MPN tubes would support different level of TMAO reduction and yield different final pHs. In order to study the relevance of this observation, we included a variety of acid-base indicators in some tests.

Resuscitation of seawater-injured E.coli. In early experiments, we transferred laboratory cultures of E.coli to artificial seawater and incubated them at 35 deg. Then we used media of various formulations to enumerate the survivors. Later we collected some naturally contaminated saline waters from San Francisco Bay and repeated our resuscitation experiments.

Personnel. These experiments were performed by technicians Rosalind Lum and Joyce Fujii, visiting graduate student Elaine Dimartines. and several undergraduate volunteers.

FINDINGS

Detection and enumeration of E.coli with "Colitag-S." Excellent agreement was found between "Colitag" and standard methods in a large variety of shellfish at all times of the year. This was true of California samples collected by the California State Department of Health Services, and also of grocery store samples from both California and out-of-state. The superior speed, convenience, specificity, and sensitivity of "Colitag" were noteworthy. The TMA production removes interference by acids produced from endogenous glycogen. There is no interference from endogenous fluorescence or glycosidase activity. Klebsiella, an interfering fecal coliform in southern waters, does not react. Nothing was gained by the addition of glycosidase substrates (ONPG & MUG) used in some other E.coli tests. Thus they were not used. No correlation was found between the extent of TMAO reduction and the presence of bacteria other than E.coli. Thus the use of pH indicators was abandoned.

Need for Additional Work: This work should be confirmed in other parts of the country with local shellfish.

Resuscitation of seawater-injured E.coli. Seawater injury of laboratory cultures at 35 deg was effectively treated by including sodium pyruvate in the growth medium. However a very limited number of field studies failed to confirm this result. We believe that this indicates (1) That laboratory cultures of E.coli are much more susceptible to seawater injury and subsequent resuscitation than are those in natural fecal contamination, or (2) That in the San Francisco Bay samples, all the susceptible E.coli are already dead, and that the survivors are a very hardy subpopulation.

Need for Additional Work: We suggest that further field studies be conducted in saline waters with fresher fecal contamination. Under these conditions, the use of pyruvate tray enable public health officials to use E.coli as a fecal indicator, and to do so with as much confidence in the marine as in the aquatic environment.

EVALUATION

The primary goal, the development and validation of a simple, rapid E.coli test far shellfish tissues, was very effectively attained. The secondary goal of improving resuscitation of seawater-injured E.coli yielded an exciting lead with the discovery of pyruvate-enhanced resuscitation. However, it should be tested further in waters with higher levels of fecal contamination than those of San Francisco Bay.